

Angiopoietin-1 Improves Outcome in Traumatic Spinal Cord Injury: Dynamic Contrast Enhanced MRI, Neurobehavioral, and Biochemical Studies

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Abstract: Traumatic spinal cord injury (SCI) results in immediate disruption of the blood spinal cord barrier (BSCB). This disruption initiates changes in blood flow, intraparenchymal hemorrhage, generation of ischemic environment and inflammatory response. The combination of these events results in the generation of an inhospitable environment for repair or regeneration of damaged spinal tracts following injury. The attenuation of the altered or compromised spinal cord vasculature is an area for therapeutic intervention. One particular agent that has been demonstrated to stabilize the disrupted vasculature is Angiopoietin-1 (Ang-1). This protein plays a critical role in vascular remodeling and maturation into functional blood vessels, along with endothelial cell sprouting. Overexpression of Ang-1 results in leakage resistant blood vessels. Current evidence indicates that Ang-1 stabilizes the vasculature through the upregulation of tight junction proteins between endothelial cells. The purpose of this study is to examine the temporal changes in the vascular permeability in SCI in response to Ang-1 treatment using dynamic contrast enhanced (DCE) MRI in parallel with neurobehavioral assays. Western analysis for tight junction proteins was performed and correlated with the DCE results.

Methods: A total of 50 adult male Sprague-Dawley rats, each weighing between 300 to 350 g, were used in these studies. All animals underwent surgery under isoflurane anesthesia in which they received a laminectomy. Animals in the injured groups received a moderately severe contusion at level T7 using the Infinite Horizon Impactor. The SCI rats were assigned to two groups: treatment with Ang-1 (1 µg/ml) & adeno-associated virus (AAV) engineered to express Ang-1 (n=25) and treatment with vehicle and AAV-LacZ (n=25). Ang-1 or control treatment was administered acutely after injury via direct microinjection into and around the lesion epicenter using a Picospritzer II apparatus. The concentration of the AAV-Ang-1 and or viral control (AAV-LacZ) was 3×10^{12} u/ml. An 11x35 mm implanted RF coil was positioned above the site of injury and was inductively coupled to an external coil for improved signal-to-noise ratio. Prior to each MRI session, a battery of neurobehavioral assays were performed to assess the animals' neurobehavioral condition. Animals were further divided into groups harvest at 48hrs, 14 days and 56 days to assess acute, sub-acute, and chronic time points. MRI scans were performed at these time points using a Bruker 7T scanner. Multi-slice RARE images were acquired with a rare factor of 4 and TE₁/TE₂/TR of 21.2/63.6/3150 ms. A total of 35 contiguous and interleaved 1 mm thick axial images with a square FOV of 2.62 cm and 256 x 256 image matrix were acquired. The RARE images were inspected for visualizing the lesions. Dynamic contrast imaging (DCE) was performed to determine the integrity of the blood spinal cord barrier. The permeability of the BSCB is determined by the Kps parameter, which is indicative of the amount of gadolinium (Gd) leakage. Differences in Kps values determined by DCE, as well as behavior measures and Western analysis for tight junction proteins for both treatment groups were evaluated using Student's t-test.

Results: Our data indicates that the Ang-1 treatment significantly reduces BSCB permeability at 48hrs and 56 days post-injury determined by DCE imaging. Our study also indicated that open field Basso, Beattie, and Bresnahan (BBB) locomotor assessment showed a significant improvement in Ang-1 treated animals compared to viral control treated group. An important observation from our data is that in the chronic time points the treatment with Ang-1 did not elicit or exacerbate mechanical allodynia compared to viral controls determined by von Frey assessment. When we examined the possible mechanism behind Ang-1 treatment, our results indicate that the attenuation of BSCB permeability is driven by the increased expression or preservation of the tight junction proteins occludin around the injury site.

Conclusions: Our study indicates that Ang-1 administration following spinal cord injury is a promising SCI intervention for improved functional recovery. Our study also determined, that promoting vascular stability after SCI results in improved BSCB permeability through the increase expression of tight junction protein occludin. The expression of other tight junction proteins is currently being investigated after Ang-1 treatment. A significant observation is the lack of mechanical allodynia in the treated animals. This is significant since mechanical allodynia is an important consequence of SCI and treatment with other vascular modulators such as VEGF result in this devastating consequence.

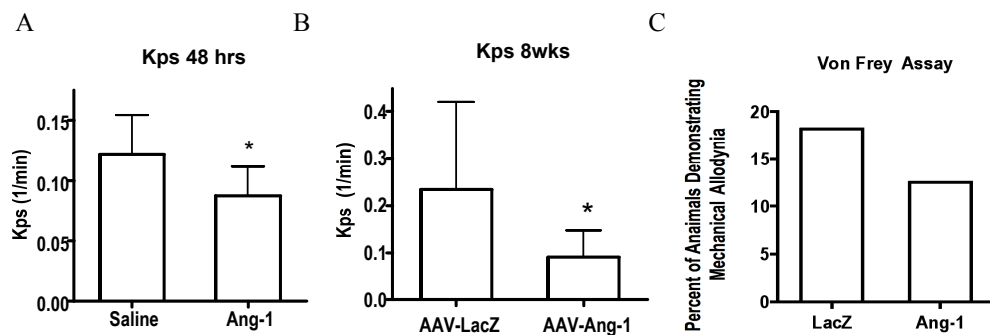


Figure 1. The combination of Ang-1 (1 µg/ml) and AAV-Ang-1 treatment appears to show decreasing Kps at (A) 48 hrs (p=0360), (B) 56 days (p=0513). Von Frey assessment for allodynia between Ang-1 treated animals compared to viral controls demonstrate no exacerbation of the development of mechanical allodynia.

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